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(71) Applicant: THE BETH ISRAEL HOSPITAL ASSOCIATION [US/US]; 330 Brookline Avenue, Boston, MA 02215 (US).

(72) Inventor: FOSSEL, Eric, T. ; 66 Priscilla Road, Chestnut Hill, MA 02167 (US).

(74) Agent: LORUSSO, Anthony, M.; Lorusso & Loud, 440 Commercial Street, Boston, MA 02109 (US).

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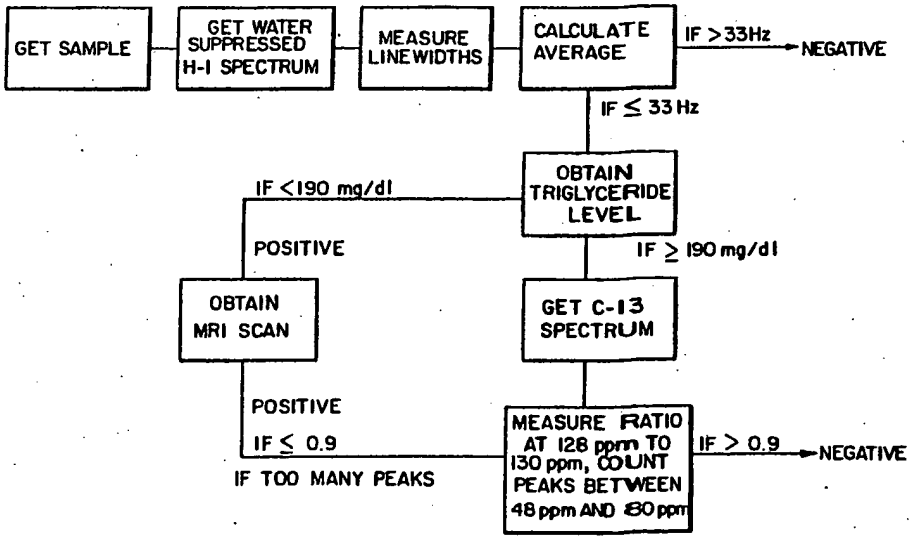
(54) Title: PROCESS FOR THE DETECTION AND LOCALIZATION OF CANCER

(57) Abstract

A method is disclosed for the detecting and localizing cancer using nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI). Specifically, NMR parameters for protons of lipid methyl and/or methylene groups are determined and compared against a corresponding value for healthy patients. In the preferred embodiments, an NMR spectrometer apparatus is employed to provide a spectrum for non-water components of blood, blood serum or blood plasma and the width of the methyl and/or methylene groups is measured at half-height as a determination of spin-spin relaxation time (T_2) which is the parameter used for purposes of comparison with healthy controls. The water proton signal is suppressed where necessary in order to obtain a suitable spectrum for the non-water component protons. In the event that a positive reading is obtained at the proton NMR stage, the level of plasma triglycerides is determined and if it is normal the patient is diagnosed as having cancer and subjected to whole body MRI to determine the location of malignancies. If however, the triglyceride level ishigh, the patient's body fluid sample is further subjected to C-13 nuclear magnetic spectroscopy. The resonance line of the plasma C-13 discriminates between true and false positive results from the proton NMR reading and determines the presence or absence of cancer in the patient. If cancer is detected at this step the patient is subjected to whole body magnetic resonance imaging to locate malignancies.



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PROCESS FOR THE DETECTION AND LOCALIZATION OF CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part application of prior pending application U.S. Serial No 418,182, filed as a continuation-in-part of USSN 325,773 on October 6, 1989, filed as a file wrapper continuation of USSN 262,073, now abandoned, filed as a file wrapper continuation of USSN 188,752, now abandoned, filed as a file wrapper contiunation of USSN 036,943, now abandoned, filed as a divisional of U.S. Serial No. 833,840 to Eric T. Fossel, filed February 26, 1986, now abandoned.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

The funding for work described herein was provided by the Federal Government, under a grant from the Department of Health and Human Services. The Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method for the detecting and localizating cancer in a living patient.

Prior Art

Methods utilizing the techniques of nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) to aid in a clinical diagnosis of cancer are well known in the prior art.

Damadian was the first to propose a medical use for NMR. He suggested it be used for detecting malignancy in tissue. See R. Damadian, "Tumor Detection by Nuclear Magnetic Resonance," Science 171:1151-1153 (1971). U.S. Patent 3,789,832 issued to Damadian covers an apparatus and method for applying nuclear magnetic resonance to surgically

removed specimens to measure T_1 and T_2 for proton relaxation times, which values, compared to values for healthy tissue, were taken as a means of diagnosing cancer. U.S. Patent Nos. 4,411,270 and 4,354,499 issued to Damadian cover an apparatus and method for cancer detection with NMR imaging and scanning of whole-body specimens.

A number of other investigators also reported that nuclear magnetic resonance relaxation times (T_1) for water protons in organs of tumor-bearing animals have higher values than the corresponding T_1 for water structure in organs of healthy animals. See Frey et al, J. Natl. Cancer Inst. 49, 903 (1972); Inch et al, J. Natl. Cancer Inst. 52, 353 (1974); Iijima et al, Physiol. Chem. and Physics 5, 431 (1973); and Hazlewood et al, J. Natl. Cancer Inst. 52, 1849 (1974).

Today, despite uncertainty regarding the mechanistic details, it is well known that biophysical changes which occur in malignant cells often alter the proton NMR signal. See D.G. Taylor et al, "A review of the Magnetic Resonance Response of Biological Tissue and Its Applicability to the Diagnosis of Cancer by NMR Radiology," Computed Tomography, 5:122-133 (1981). Such changes form the physical basis for detecting tumors by proton NMR imaging. See R. Zimmerman et al, "Cerebral NMR: Diagnostic Evaluation of Brain Tumors by Partial Saturation Technique with Resistive NMR," Neuroradiology 27:9-15 (1985) and K. Ohtomo, "Hepatic Tumors: Differentiation by Transverse Relaxation Time (T_2) of Magnetic Resonance Imaging," Radiology 155:421-423 (1985).

Proton NMR studies on excised tumors, as well as on plasma and serum, from experimental animals and patients have often shown differences in the relaxation parameters T_1, T_2, and T_2^* (T_2^* being a combination of T_2 from intrinsic relaxation and relaxation induced by magnetic field inhomgenieties) as a function of malignancy. Such findings have been reported by the following:

L. McLachlan, "Cancer-induced Decreases in Human Plasma Proton NMR Relaxation Rates," Phys. Med. Biol. 25:309-315 (1980):

F. Smith et al, Nuclear Magnetic Resonance Imaging of the Pancreas," Radiology 142:677-680 (1982);

P. Beall et al, "The Systemic Effect of Elevated Tissue and Serum Relaxation Times for Water in Animals and Humans with Cancers," NMR Basic Principles and Progress, P. Diehl et al, Eds., 19:39-57 (1981)

R. Floyd, "Time Course of Tissue Water Proton Spin lattice Relaxation in Mice Developing Ascites Tumor," Cancer Res. 34:91 (1974);

C. Hazlewood et al, "Relationship Between Hydration and Proton Nuclear Magnetic Resonance Relaxation Times in Tissues of Tumor Bearing and Nontumor Bearing Mice: Implications for Cancer Detection," J. Natl. Cancer Inst. 52:1849-1853 (1974); and

R. Klimek et al, "A Discussion of Nuclear Magnetic Resonance (NMR) Relaxation Time of Tumors in Terms of Their Interpretation as Self-organizing Dissipative Structures, and of Their Study of NMR Zeugmatopgraphic Imaging," Ginekol Pol. 52:493-502 (1981).

However, due to extensive overlap of groups and small differences between the means of groups, these methodologies are not clinically useful.

While most of the prior art mentioned above suggests using NMR to analyze tissue, it is also known that body fluids are subject to such analysis, as discussed by Beall et al., supra.

The cited prior art NMR methods for detecting malignancy rely on the interpretation of the composite NMR signal arising from all protons in the tissue or blood derived samples. This composite signal is dominated by the protons of water, obscuring the NMR signal from other proton-containing constituents of the sample. Indeed, the

prior art believed that the apparent correlation between malignancy and observed changes in NMR parameters was due to changes in water structure," quoting Frey et al., supra.

In other applications of proton NMR spectroscopy, it was known to suppress the signal from the solvent (such as water), in a sample. It was discovered that the components of the NMR spectrum which have significant predictive value may be masked by other materials in the sample. By eliminating the water signal, the previously masked spectrum of these components was revealed. In co-pending application of Eric T. Fossel, entitled "Process for the Detection of Cancer Using Nuclear Magnetic Resonance," U.S. Serial No. 303,586, filed January 27, 1989, the teachings of which are incorporated herein by reference, the aforementioned discoveries were incorporated into a reliable method of diagnosing cancer in a living patient.

In accordance with that invention, a sample of a patient's bodily fluid is subjected to nuclear magnetic resonance spectrum. A resonance line generated by a non-water component of the sample is selected, and the full width of this resonance line at a given height, e.g., at half its height, is measured. The full width so measured has proved to be a statistically reliable measure of the presence or absence of cancer in a patient.

The above-described test of water-suppressed proton NMR of plasma discriminates between persons with untreated cancers and others with better than 90% accuracy. As such, the test was widely acclaimed as one of the most important inventions of the decade. No prior non-invasive test for Cancer has approached that level of accuracy. False positive results, however, have been obtained.

In the co-pending application of Eric T. Fossel, entitled "Process for the Detection of Cancer Using NMR (Carbon 13)," USSN 295,746, the teachings of which are incorporated herein by reference, it was shown that the major source of false positive results is high levels of

plasma triglycerides. That invention teaches a non-invasive method and apparatus with improved accuracy over prior non-invasive methods to determine the presenc of cancer in a living patient.

In accordance with that invention, the triglyceride level is measured on those patients with a positive result on the proton NMR screening test. A normal triglyceride level confirms the cancer diagnosis; however, the fluid samples of patients with high triglyceride levels are subjected to C-13 NMR. An abnormal result in that test confirms the cancer diagnosis, whereas a normal result indicates that the prior diagnosis was a false positive.

In the co-pending application of Eric T. Fossel entitled "Apparatus and Method for Detection Cancer Using Nuclear Magnetic Resonance," USSN 418,182, filed October 6, 1989, the teachings of which are incorporated herein by reference, it was shown that diagnoses could be made through an automated process which incorporated both proton NMR and C-13 NMR spectroscopy.

In accordance with that invention, an apparatus was designed that automated the process for diagnosing cancer using NMR. The apparatus has a spectrometer component capable of taking water suppressed proton NMR and C-13 NMR readings of a fluid sample. Additionally, the apparatus has computer means for processing the proton and C-13 readings and for obtaining a numerical value corresponding to those readings. The apparatus further comprises memory means for storing a set of standard of normal values and comparing those stored values to values obtained from the NMR. The computer programs which direct its function, analyzes the data and yields a diagnosis with a great degree of accuracy.

Diagnosis is just the first step in cancer treatment. Identification of particular malignancies must follow. Nothing in the prior art teaches a method which uses a process of obtaining NMR spectra, interpreting such spectra, making diagnoses and then when positive diagnoses of cancer

are made, locating the cancer through the use of magnetic resonance imaging (MRI). The coupling of a NMR apparatus to obtain initial diagnoses and whole body MRI scanning leading to a extremely accurate and specific diagnoses of cancer is highly desireable.

SUMMARY OF THE INVENTION

In accordance with the present invention, a method for detecting and locating cancer was developed using a nuclear magnetic resonance apparatus and a magnetic resonance imaging system. The method and apparatus is capable of generating a diagnosis using proton NMR spectroscopy, factoring levels and if necessary further subjecting the sample to C-13 NMR spectroscopy. Should a true positive reading occur at either the triglyceride or C-13 steps, the patient is then subjected to whole body MRI to identify malignancies.

In the preferred embodiments, the sample fluid is blood, spinal fluid, or bone marrow plasma; blood is especially advantageous. The component of interest is lipidic, and is preferably from the methyl and methylene groups of the lipoprotein lipids.

Accordingly, an object of the present invention is to provide an accurate method of diagnosis using a NMR spectroscopy process to detect the presence of cancer in a living patient and when there is a true positive diagnosis of cancer, immediately using an MRI scanner to identify and locate malignancies.

Other objects and advantages of the invention will become apparent from the descriptions of the drawings and the invention which follow.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a typical 360 MHz NMR spectrum for the non-water components (water-suppressed) of a plasma sample from a healthy control obtained in accordance with the first stage of the present invention;

FIG.2 is an NMR spectrum for the same plasma sample from which the spectrum of FIG. 1 was obtained, using the same equipment and pulse frequency, except without water suppression;

FIG. 3 is an expanded view of the methyl and methylene region of the reading of the sample of FIG. 1;

FIG. 4 is an expanded view of the methyl and methylene region of an NMR spectrum for a plasma sample for a patient with an untreated malignancy;

FIGS. 5A and 5B are C-13 NMR spectra of a plasma sample the olefinic region for a normal control and an untreated cancer patient, respectively, obtained in accordance with the first stage of the present invention;

FIGS. 6A and 6B are views of the C-13 NMR spectral region between 10 ppm and 90 ppm, with particular inclusion of the region between 48 ppm and 80 ppm, of the plasma samples shown in FIGS. 5A and 5B for a normal control and an untreated cancer patient, obtained in accordance with the first stage of the present invention;

FIG. 7 schematically illustrates the first stage of the apparatus of the present invention;

FIG. 8 shows the results of a study using the method of the present invention;

FIG. 9 shows a flowchart diagramming the operation of the invention;

FIG. 10A shows a flowchart for shimming, a task carried out by the first stage of the apparatus which ensures reproducible results from water suppressed readings;

FIG. 10B shows a sample program for shimming, corresponding to the proton spectrum step of the flowchart in FIG. 10A.

FIG. 10C show a sample program for shimming, corresponding to a carbon-13 spectrum;

FIG. 10D shows a sub-program of step 8, RJ FQSET, of the shimming program shown in FIG. 10C;

FIG. 10E shows a sub-program of step 2, RJ TUNE, of the program shown in FIG. 10B; and

FIG. 10F shows a sub-program in FIG. 10B.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

At the outset, the invention is described in its broadest overall aspects with a more detailed description following. The present invention is a method for detecting the presence and location of cancer in a living patient. In accordance with the invention, a sample of a patient's bodily fluid is subjected to proton nuclear magnetic resonance spectroscopy to generate a nuclear magnetic resonance spectrum. Since components of the NMR spectrum which have significant predictive value may be masked by other materials in the sample, the masking is eliminated to produce an informative NMR spectrum. A resonance line generated by a non-water component of the sample is selected and a predetermined widths, most preferably the full width, of the resonance line at a predetermined height, e.g., at half its height, is measured to provide a reliable measure for the screening of cancer in the patient. The theory or the above procedure is described in co-pending application, United States Serial No. 303,586, the teachings of which are incorporated herein by reference.

In practice, a component of the NMR apparatus makes the measurement, or measurements in the event that more than one resonance line is selected, and compares the average value obtained to a stored value which is indicative of the normal value; i.e., for a cancer-free person.

A positive reading may indicate the presence of cancer in the patient, or it may be a false positive reading. It has been discovered that a major source of false positive

r adings are persons with high levels of plasma triglycerides. Accordingly, if the measured resonance line value is greater than the stored normal value, the program will diagnose the patient as cancer-free. However, if the measured value is less than the stored value, the program will direct the apparatus to obtain a measure of the patient's triglyceride level.

In order to differentiate between true and false positive readings, those samples with elevated triglyceride levels will be subjected to C-13 NMR spectroscopy. This will result ina s diagnosis. The false positive results due to hypertriglyceridemia and, conversely, the presence of cancer in the patient, can be reliably determined from certain features of the C-13 spectra.

Should a positive cancer detection be obtained either with a positive proton NMR spectroscopy reading and normal triglyceride level or a positive proton NMR spectroscopy, high triglyceride level and a positive C-13 result, the patient is then subjected to to whole body MRI scanning to determine the malignancy's location.

In one embodiment of this invention, proton NMR spectroscopy is performed initially on the sample to be tested. The water suppressed proton NMR spectrum obtained on human blood plasma is dominated by the resonances of the plasma lipoprotein lipids. Without water suppression, these non-water resonances are virtually overwhelmed by the water. Signal averaging allows observation of resonances of some moieties associated with non-water bodily fluid components, at high magnetic fields, even in the presence of the water resonance. The capability of modern NMR spectrometers to suppress nearly completely the water proton resonance facilitates this reading. The water suppressed proton NMR spectrum of plasma is essentially that of plasma lipoproteins and a few low molecular weight molecules. The plasma protein protons are obscured because they comprise a

broad smear of unresolved resonances. The sharper resonances of the more mobile lipoprotein protons are superimposed on this broad background.

The NMR spectrometer of the present invention operates on any lipid-containing body fluid. Whole blood, serum or plasma may be used. While the test may be performed on any such lipid-containing body fluid, work to date has focused on blood plasma. In blood, the lipids, including cholesterol, triglycerides, and phospholipids, are present in the form of lipoproteins. The test for cancer will typically be performed in vitro, preferably on serum or plasma.

The selected fluid of a person to be screened for cancer is exposed to both a magnetic filed and radio-frequency energy to generate a nuclear magnetic resonance signal which is then processed by the NMR spectrometer which obtains a value for a selected parameter, e.g., $W_{1/2}$, for lipid methyl and/or methylene protons. A relatively broad range of proton frequencies may be employed, e.g., 60 MHz and higher; 360 MHz or above is a preferred frequency. If cost is not a factor, 500 MHz may be the preferred frequency.

FIG. 1 shows a water suppressed proton spectrum of a healthy control, and FIG. 2. shows a proton spectrum of the same sample without water suppression. The truncated resonance line of water is denoted A in FIG. 2. The resonance lines between 0.5 and 2 ppm (parts per million of resonance frequency) arise from the methyl and methylene groups of the lipoprotein lipids. An expanded view of this region of the proton spectrum is shown in FIG. 3 for a normal control and in FIG. 4 for a patient with untreated malignancy. Accordingly, in its preferred embodiments, the present invention uses one of a number of conventional water suppression techniques, i.e., techniques for suppression of the water proton NMR signal. Numerous techniques have been devised to suppress the water proton NMR signal in other

contexts. These may be broadly divided into two categories: (1) thos that aim not to excite the water proton signal, e.g., rapid scan correlation spectroscopy and the selective excitation technique, and (2) those that arrange for the water proton magnetization to be extremely small at the time the observed radio frequency (rf) pulse is applied, e.g., the inversion recovery technique and saturation. These and other solvent suppression techniques are described by P.J. Hore in "Solvent Suppression in Fourier Transform Nuclear Magnetic Resonance," Journal of Magnetic Resonance 55:283-300 (1983) and the references footnoted therein. Although the water suppression technique is preferred when using a conventional NMR apparatus due to its inability to distinguish between the signal of the solvent protons and those of the moiety or species of interest, a sufficiently sensitive apparatus would eliminate the need for water suppression altogether.

The moiety resonance linewidth at a predetermined height, most preferably half-height, e.g., methyl and methylene groups, associated with the lipids of plasma lipoprotein is the variable of interest. Full width at half-height $W_{1/2}$ (linewidth) of an NMR resonance line is inversely proportional to the apparent spin-spin relaxation time (T_2^*),

i.e. $W_{1/2} = \frac{1}{T_2^*}$.

The detected value for the selected parameter is compared with the corresponding parameter for the healthy controls, this comparison is most preferably done by computer means within the NMR apparatus. In a preferred embodiment, values for methyl and methylene protons are averaged and an average value of 33 Hz or less at a proton frequency of 360 MHz (8.45T) pr 400 MHz (9.40T) is taken as an indication of malignancy.

If a positive reading is obtained from th water suppressed proton NMR spectrum of a patient's plasma, a second level of testing is preformed to confirm the diagnosis. First, a conventional test, commonly called a triglyceride analysis, is performed to determine the patient's triglyceride level. If the triglyceride level is normal, the positive reading from the water-suppressed proton NMR spectroscopy is a true positive and indicates the presence of cancer in the patient, in which case, the patient would then be subjected to whole body MRI scanning to ascertain the malignancy location.

If, however, the triglyceride level is above normal, the NMR spectrometer apparatus is directed, most preferably by computer means, to obtain a C-13 NMR spectrum of the patient's plasma sample, which is already available because of the earlier proton NMR spectrum, in order to differentiate between true and false positive results. False positive results due to hypertriglyceridemia can be reliably distinguished from true positive results by substantial differences in features of C-13 spectra. Accordingly, the plasma sample already obtained from the suspect patient to be diagnosed is exposed to a magnetic filed and radio frequency energy to generate a C-13 nuclear magnetic resonance spectrum.

Initially, the olefinic region, 120-140 ppm, of the spectrum is examined. Two peaks will appear, one at approximately 128-129 ppm and another at approximately 130-131 ppm, about 2 ppm apart. The ratio of the resonance at the general region of 128 ppm to that at 130ppm is determinative of cancer. In readings of plasma from normal controls and from persons with non-cancer disease, the ratio of the height of those two resonances (128/130 ppm) is 0.9 or greater, i.e. the resonance peak at 128 ppm is approximat ly equal to or of greater height than that at 130 ppm. The heights of the peaks are measured by computer from the center of the baseline noise to the top of the peak. In

readings of plasma from patients with untreated cancer, the ratio of the peak heights is less than 0.86, or the resonance peak at 130 ppm is greater in height, by at least 5%, than that at 128 ppm. It should be noted that in patients with hypertriglyceridemia, the ratio of the resonances' (128/130) height is the same as normal control values. Accordingly, the computer of the NMR spectrometer apparatus will calculate the ratio of the peak heights already measured, and if the ratio is greater than a stored value will diagnose the patient as healthy, but otherwise will render a diagnosis of cancer. In a preferred embodiment, the stored value is 0.9. A diagnosis of cancer would mean that the patient is then subjected to MRI scanning as described above.

FIGS. 5A and 5B show the olefinic regions of spectra taken at 125.76 MHz with broadband proton decoupling from a normal control patient and an untreated cancer patient. In FIG. 5A, the ratio is 1.14 in the normal control patient and in FIG. 5B the ratio is 0.70 in the untreated cancer patient. In the patients with hypertriglyceridemia that were studied, the ratio ranges from 1.05 to 1.68.

The changes in the olefinic region of the spectra of untreated cancer patients can be explained by increases or decreases in polyunsaturated fatty acid chains in the lipids. The levels of oleic and linoleic acid are particularly indicative.

Oleic acid is a monounsaturated fatty acid made by the human body. Linoleic acid is a diunsaturated fatty acid and is not made by the human body, but obtained by consumption. Dietary fatty acids include polyunsaturated acids, such as linoleic acid. A resonance peak in the general region of 128-129 ppm evidences only linoleic acid in the patient. A resonance peak in the general region of 130-131 ppm evidences both oleic and linoleic acid in the patient.

It was discovered that the height of those resonance peaks, relative to each other, are affected by certain conditions of the patient. For example, persons with high triglyceride levels usually have a high ratio of linoleic acid to oleic levels. Patients with untreated cancer are found to have low levels of linoleic acid in their bodies, presumably because cancer causes oxidation of polyunsaturated fatty acids, including linoleic. This is consistent with the hypothesis that lipids are oxidized by hydroxyl free radicals in cancer patients since polyunsaturated fatty acids are most susceptible to oxidation.

Accordingly, if the subject patient has both high triglycerides and untreated cancer, the resonance peak at 130 ppm will be higher, reflecting the decreased linoleic acid in both peaks. If however, the peak at 128 ppm is not shorter than that at 130 ppm by more than 7% , no depression, or an insignificant depression, of linoleic acid levels has occurred, and the positive result obtained from the proton NMR spectra is confirmed as a false positive. In that case, the NMR spectrometer apparatus renders a diagnosis of no cancer present.

In addition, the spectral region between 48 ppm and 80 ppm is far more complex in untreated cancer plasma than in normal control or hypertriglyceridemia plasma. By "more complex" is meant that there are more resonance peaks in the region. A resonance peak is counted by the program if its height is at least 50% greater than that of the background noise during a normal testing period. As those skilled in the art will know, the longer data is collected, the better the resolution. FIGS. 6A and 6B show this region for normal control and untreated cancer plasma, respectively. These spectra were obtained at 125.76 MHz using a 5 mm sample tube and 14 hour accumulations. C-13 spectra with adequate information can also be obtained at 90.5 MHz in 10 mm or

longer sample tubes. Of course, changes to the parameters of the test procdure will be evident to those skilled in th art.

These parameters include the size of the sample tube, the pulse width, the pulse repetition rate, and the exponential multiplication of the free induction decay by different factors. For example, it is obvious to those skilled in the art that the larger the sample tested, the faster spectra of adequate quality will be obtained. Other changes to the conditions given here will be evident to those skilled in the art.

C-13 spectroscopy or MRI scanning could be performed initially on a patient as a method to diagnose the presence of cancer, without first obtaining a proton NMR spectrum as described above. While generally it takes 30 seconds to obtain a proton NMR spectroscopy , a C-13 spectrum may take anywhere from one to fifteen hours, therefore it is both time consuming and expensive to perform. Accordingly, in a preferred embodiment, C-13 spectroscopy is used to verify the positive results obtained from the proton NMR spectra and illuminates statistically and clinically significant differences in a plasma C-13 spectra between true and false positive results from the proton water suppressed NMR spectroscopy test. Whole body MRI scanning is similarly expensive. The use of C-13 NMR spectroscopy prior to MRI scanning would avoid the use of another piece of machinery as well as needlessly subjecting patients with false positive readings to additional medical procedures.

In the preferred embodiments, an NMR spectrometer with a magnet at constant field strength is used and the NMR signal is Fourier transformed, with the the NMR parameters of interest being the full linewidth at half-height for proton resonances of methyl and methylene groups and the C-13 resonances of linoleic acid.

As noted in patent application, U.S. Serial No. 833,840, correct sample preparation and execution is essential to carry out a successful measurement of a plasma sample. Blood is collected in tubes containing 70 microliters of a solution of 15% Na_2EDTA. Blood was maintained at 4°C until centrifugation. Plasma was separated and stored at 4°C until NMR analysis. Plasma samples were never frozen because freezing destroys lipoprotein lipid structural integrity. Samples which showed any visible sign of hemolysis were excluded.

All spectra were obtained at 20-22°C and magnetic field strengths of 360 MHz or greater. The samples were shimmed individually by computer on the area of the proton free induction decay until the full width at half height of the water resonance was 4 Hz or less. Of course, careful shimming is an assumed component of good NMR laboratory technique.

It can be seen that of those experimental conditions, temperature and shimming are not as critical with the C-13 NMR spectroscopy because a measurement of the linewidth is not taken. Of course, the field strength used will determine the time required to obtain a spectrum. In addition, an accurate diagnosis requires careful review of a patient's medical records.

FIG. 9 show a flowchart of the operation of the process in which a sample of bodily fluid is obtained and is submitted to NMR spectroscopy to obtain a water-suppressed H-1 spectrum. The apparatus then selects and measures resonance lines and finds an average linewidth which it compares with the value of 33 Hz, a predetermined normal value. If the average linewidth is greater than

33 Hz, the apparatus will yield a negative diagnosis; otherwise, it will obtain a measure of the triglyceride level in the patient. If the triglyceride level is less than 190 mg/dl then it will render a positive diagnosis for cancer and the patient will be subjected to whole body MRI

scanning. If, however, the triglyceride level is greater than 190 mg/dl then the apparatus will obtain a C-13 spectrum of the sample. It will then analyze the C-13 spectrum by measuring the ratio of the peak at 128 ppm to the peak at 130 ppm and by counting the number of peaks in the range 48 ppm to 80 ppm. If the ratio is 0.9 or greater, the apparatus will yield a negative diagnosis; however, if the ratio is less than 0.9 or there is an abnormally high number of peaks in the range of 48 ppm to 80 ppm then the apparatus will diagnose the patient as having cancer and as stated above the patient is then subjected to MRI scanning to locate malignancies. The above diagnostic procedure comprising factoring the triglyceride levels and C-13 NMR spectroscopy is described in co-pending application of Eric T. Fossel entitled "Process for the Detection of Cancer Using Nuclear Magnetic Resonance (C-13)", United States Serial No. 325,773 filed March 20, 1989, the teachings of which are incorporated herein by reference.

Referring to FIG. 7, there is illustrated a nuclear magnetic resonance spectrometer 2 which in the preferred embodiment is capable of performing proton and C-13 NMR spectroscopy and which is preferably, but not necessarily, of the type that suppresses the NMR signal of water. In order to produce reproducible H-1 or C-13 spectra, it is necessary to shim. The procedure for shimming is shown as a flowchart in FIG. 10A in which a sample is placed into the machine, the temperature is stabilized, the shimming parameters called, the receiver gain is adjusted, shimming is performed, the water frequency is found for H-1 water suppressed spectra, the receiver gain is adjusted on the suppressed proton frequency induction decay area for the water suppressed spectra, data is acquired, a file is written, and the file is processed. FIG. 10B show a program (written using the Bruker DISNMR software package) used to automatically perform the procedures depicted in FIG. 10A. Fig 10C shows a sample program for shimming, corresponding

to a carbon-13 spectrum. FIG. 10D shows a sub-program of step 8, RJ FQSET, of the shimming program shown in FIG. 10C. FIG. 10E shows a sub-program of step 2, RJ TUNE, of the program shown in FIG. 10B; and FIG 10F shows a sub-program of step 6, RJ FQSET, of the program shown in FIG. 10B. The spectrometer 2 is adapted for examination of a sample 4, which in this example is human blood plasma within a test tube 6. The above procedure is described in co-pending application to Eric T. Fossel entitled: "Apparatus and Method for Detecting Cancer Using Nuclear Magnetic Resonance", United States Serial No. 418,182 filed October 6, 1989, the teachings of which are incorporated herein.

In accordance with the invention, the spectrometer 2 contains means 8 for selecting at least one and preferably a plurality of NMR resonance lines in the NMR spectrum of the sample 4 and measuring the linewidth of the line or lines so selected. Preferably, the linewidth is measured at half the height of the line, but this is not necessary and linewidth can be measured at any predetermined fraction of the height of the line in question. Measurement at half of line height is preferred because this is a standard measurement carried out in the field of NMR spectroscopy. Several commercially available computer programs can be used for automatically measuring full linewidths at half height.

The means 8 of spectrometer 2 of the invention also measure selected peaks for the examination of the C-13 NMR spectra. The spectrometer 2 also is of conventional construction and includes in addition to all its other structure a means 10 for storing a value or range of values. In accordance with the invention, the spectrometer 2 also includes means 12 for comparing a linewidth which is either measured directly or derived from a plurality of such direct measurements with a value or range of values which represent the value or range of values to be expected from normal patients, i.e. patients who are free of cancer. Means 12 are also used for classifying the measured or derived

linewidths, peak heights, and number of peaks as normal (i.e. cancer-free) or abnormal (i.e. cancerous) based upon the stored information. This may be done by comparison, subtraction, or any other appropriate mathematical operation.

In a preferred embodiment, the selecting and measuring means 8 is pre-adjusted to measure the linewidths of the methyl and methylene groups of the lipoprotein lipids, and the peak heights and number of peaks in the C-13 NMR spectra. This may include suppressing the signal of water from the NMR spectrum of the sample 4, or may alternatively be done directly where the spectrometer 2 is sensitive enough to do so.

In a preferred embodiment, the linewidths of the methyl and methylene groups are averaged by the measuring means 8 to produce a composite linewidth which is the mathematical mean of the two. This composite linewidth is compared with 33 Hz, the value which is preferable stored in the means 10, by the classifying means 12. When the comparison shows that the composite linewidth is less than 33 Hz, this indicates an abnormal (i.e. cancerous) sample 4.

EXAMPLE

In this example, the method of the present invention was applied to a group of 135 patients undergoing breast biopsy for palpable and non-palpable breast lesions. For the prospective breast study, blood was collected and maintained at $4^{o}C$ until centrifugation. Blood was collected in non-siliconized vacutainer tubes containing 70 microliters of a solution of 14% Na_2EDTA. Plasma was separated and stored at $4^{o}C$ until NMR analysis. Plasma samples were never frozen because freezing destroys lipoprotein lipid structural integrity. Samples which showed any visible sign of hemolysis were excluded.

Plasma triglyceride concentrations were measured (Damon Clinical Laboratories, Westwood, Massachusetts) on all fresh plasma samples. All spectra were obtained at 21°C using an NMR spectrometer of the type described in this invention operating at 360 MHz for proton (H-1) and 90.5 MHz for carbon (C-13). Additional C-13 spectra were obtained on an 11.8 T Bruker AM spectrometer operating at 125.7 MHz. All studies were carried out in 5 mm OD sample tubes (Wilmad, Vineland, New Jersey; #507PP or #528PP). Each sample, containing 0.6 ml plasma, was shimmed individually on the area of the proton free induction decay (FID) until the full-width at half-height (FWHH) of the water resonance was 4 Hz or less. An internal quality control was found in the linewidth of the EDTA resonances. If all was well with the sample preparation and shimming, the linewidth (FWHH) of the EDTA resonances (without exponential broadening) had to be 2 Hz or less and was often between 1.0-1.5 Hz. In order to accomplish this, most H-1 probes require detuning to avoid radiation damping. The probe was detuned until the 90° radio-frequency pulse became 20 msec. In the 8.45 T spectrometer, this resulted in probe detuning of about 2 MHz. The sample was spun during shimming of the Z shim coils and during data acquisition. Our H-1 spectra were acquired using presaturation to suppress water and an inversion-recovery sequence to null any lactate methyl protons present. The presaturation pulse was 4.0 sec, with a delay of about 0.8 sec between the 180° and 90° pulse. Eight FIDs were signal averaged and then Fourier transformed following multiplication by an exponential resulting in 2 Hz line-broadening. The portion of the spectrum from 0.5 to 1.6 ppm was phased so that the baseline level at the edges of the plot was the same. This resulted in defective phasing of other (non-plotted) portions of the spectra.

C-13 spectra were obtained at 8.45 and 11.5 T signal with broadband proton decoupling by averaging between 2,000 and 28,000 FIDs depending on signal-to-noise level and

resolution desired. The sample was identical to the samples for H-1 spectra except 100 microliters of D_2O was added for field lock. It was found that a minimum of 2,000 FIDs were required to produce reliable resonance intensities. Exponential multiplication equivalent to 25 Hz line-broadening was used in the spectra obtained obtained at 8.45T.

In this study, we prospectively obtained plasma from a series of women undergoing breast biopsy. Samples were drawn prior to the biopsy, analyzed by NMR, and results were correlated with pathology reports. Two groups of patients were included in this study; 63 patients with palpable lesions and 72 patients with mammographically discovered, non-palpable lesions requiring wire localization. Results of the H-1 NMR spectroscopic evaluation are shown in FIG. 8. In both groups, benign lesions were clearly distinguished from malignant ($p<0.0001$) based upon the proton values. For those values, triangles indicate patients who also had elevated triglyceride values. The open symbols indicate samples in which the C-13 results conflicted with the proton results. Thus, for the open symbols, the sample would be changed from the benign column to the malignant column or vice versa.

The patients in the study were a group of otherwise healthy women, outpatients, referred for evaluation because of an abnormality on a routine breast examination or a screening mammogram. In this group, the sensitivity and specificity were 93% and 95%, respectively. The predictive value of a positive test was 84%, and the predictive value of a negative test was 98%. Reclassifying patients on the basis of the C-13 data raises sensitivity and specificity to 97% and 98%, respectively, and the predictive value of a positive test to 93%. These data suggest that the H-1 NMR linewidth, confirmed by a C-13 ratio, might be used asa diagnostic aid in patients with breast lesions.

There were five apparent false positive and two false negative results. Elevated triglyceride levels (265 mg/dl and 206 mg/dl) were associated with two of the five false positives. The C-13 ratio was negative in three false positives, two of which also had elevated triglycerides; and it was positive in all but one of the cases with malignant breast biopsis. The one false negative by C-13 was also negative by H-1. While patients with elevated triglyceride levels may need to be evaluated by C-13 also, not all patients with elevated triglycerides had narrowed linewidths.

The invention may be embodied in other specified forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range or equivalency of the claims are therefor intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A relatively non-invasive method for the diagnosis of the presenc and location of cancer in a living patient comprising:

a) taking a fluid sample from a patient;

b) preparing the fluid sample for NMR testing;

c) generating a proton NMR spectrum from which undesirable signals have been suppressed from the bodily fluid sample;

i) measuring a predetermined width at a predetermined height of a lipoprotein resonance line in said spectrum;

ii) classifying the predetermined width into a category of normal widths or into a category of abnormal widths as compared to a predetermined standard for which abnormal widths indicate the presence of cancer;

d) measuring the triglyceride level of the bodily fluid sample utilized when the bodily fluid sample is classified as having abnormal widths;

i) classifying the triglyceride level so measured into a category of normal levels or above normal level, for which a normal triglyceride level and an abnormal spectrum indicates the presence of cancer;

e) reporting a positive or negative cancer diagnosis;

f) subjecting patient with positive cancer diagnosis to whole body magnetic resonance imaging;

g) interpreting imaging scan to determine the presence or absence of masses; and

h) reporting the presence or absence of cancerous masses.

2. The method of claim 1, wherein the bodily fluid is blood;

3. The method of claim 2, wherein said blood sample is prepared by removing red cells therefrom;

4. The method of claim 1, wherein the undesirable signal suppressed is water;

5. The method of claim 1, wherein the predetermined width at a predetermined height is full width at half height;

6. The method of claim 1, wherein the proton resonance is above 60 MHz;

7. The method of claim 6, wherein the proton resonance is equal to or above 360 MHz.

8. The method of claim 1, wherein measuring the predetermine width at a predetermine height is accomplished by a computer.

9. The method of claim 1, wherein classifying the predetermine width is accomplished by a computer.

10. A relatively non-invasive method for the diagnosis of the presence and location of cancer in a living patient comprising:

a) taking a fluid sample from a patient;

b) preparing the fluid sample for subjecting to NMR;

c) generating a proton NMR spectrum from which undesirable signals have been suppressed from the bodily fluid sample;

i) measuring a predetermined width at a predetermined height of a lipoprotein resonance line in said spectrum;

ii) classifying the predetermined width measured into a category of normal widths or into a category of abnormal widths as compared to a predetermined standard for which abnormal widths indicate the presence of cancer;

d) reporting the predetermined width as normal or abnormal;

e) measuring the triglyceride level of th bodily fluid sample utilized when the bodily fluid sample is classified as having abnormal full widths;

i) classifying the triglyceride level so measured into a category of normal levels or above normal levels;

f) reporting the triglyceride as normal or above normal;

g) generating a C-13 nuclear magnetic spectrum from the bodily fluid sample utilized when said bodily fluid is classified as having above normal levels of triglycerides;

i) classifying the C-13 nuclear magnetic spectrum into a category of normal or abnormal spectrum, for which an abnormal spectrum indicates the presence of cancer;

h) reporting the C-13 nuclear magnetic spectrum as indicative of cancer;

i) subjecting patient with positive cancer diagnosis to whole body magnetic resonance imaging;

j) interpreting imaging scan to determine the presence or absence of masses; and

k) reporting the presence or absence of cancerous masses.

11. The method of claim 10, wherein the bodily fluid is blood;

12. The method of claim 10, wherein said blood sample is prepared by removing red cells therefrom;

13. The method of claim 10, wherein the undesirable signal suppressed is water;

14. The method of claim 10, wherein the predetermined width at a predetermined height is full width at half height;

15. The method of claim 10, wherein the proton resonance is above 60 MHz;

16. The method of claim 15, wherein the proton resonance is equal to or above 360 MHz.

17. The method of claim 10, wherein the ratio of peaks measured between the C-13 nuclear magnetic resonance 128 ppm to 130 ppm is diagnostic for cancer.

18. The method of claim 17, wherein the ratio of the peaks measured between the C-13 nuclear magnetic resonance 48 ppm to 80 ppm is determinative of cancer.

19. The method of claim 10, wherein measuring the predetermine width at a predetermine height is accomplished by a computer.

20. The method of claim 10, wherein classifying the predetermine width is accomplished by a computer.

21. The method of claim 10, wherein classifying the triglceride level is accomplished by a computer.

22. The method of claim 10, wherein classifying the C-13 nuclear magnetic spectrum is accomplished by a computer.

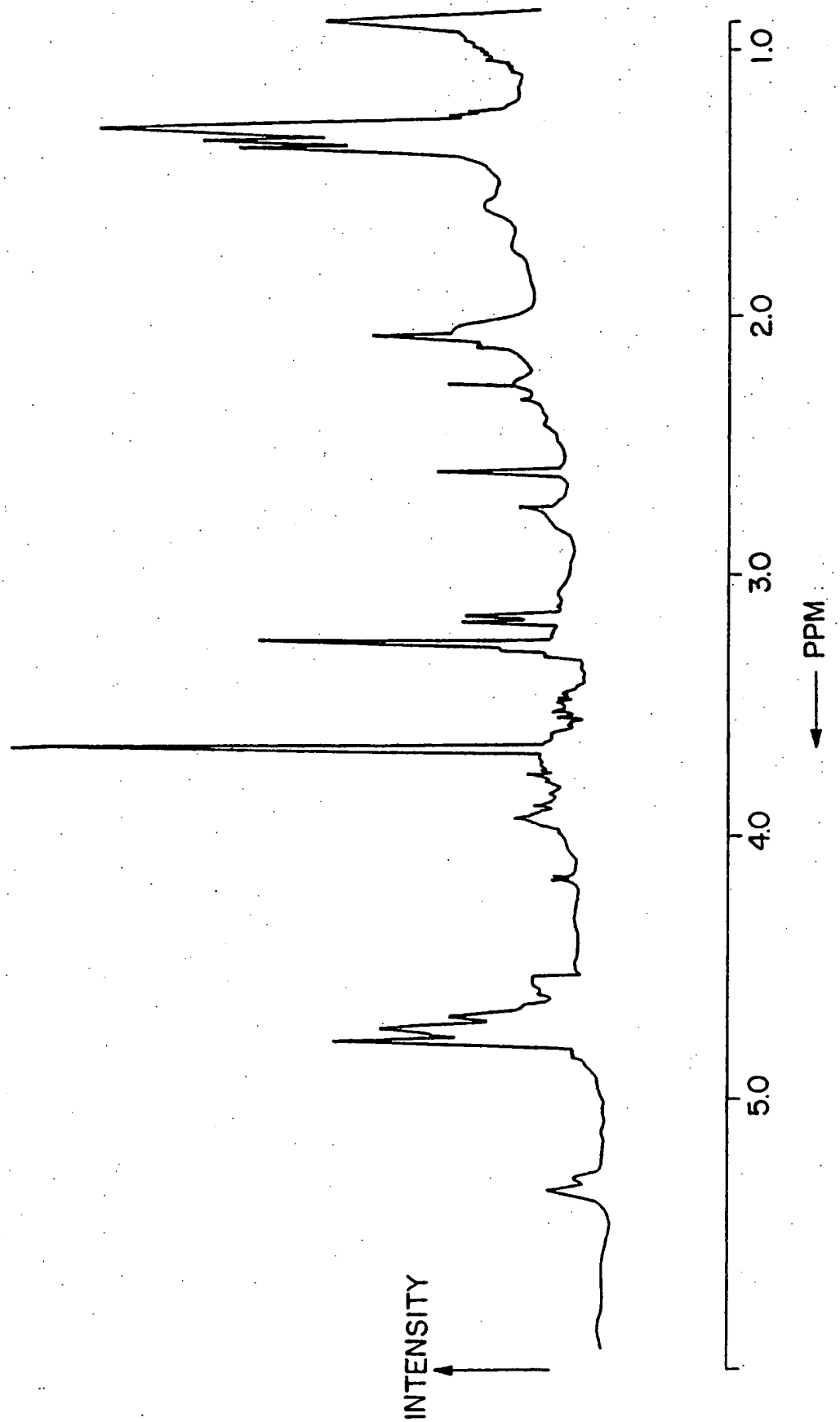


FIG. I

FIG. 2

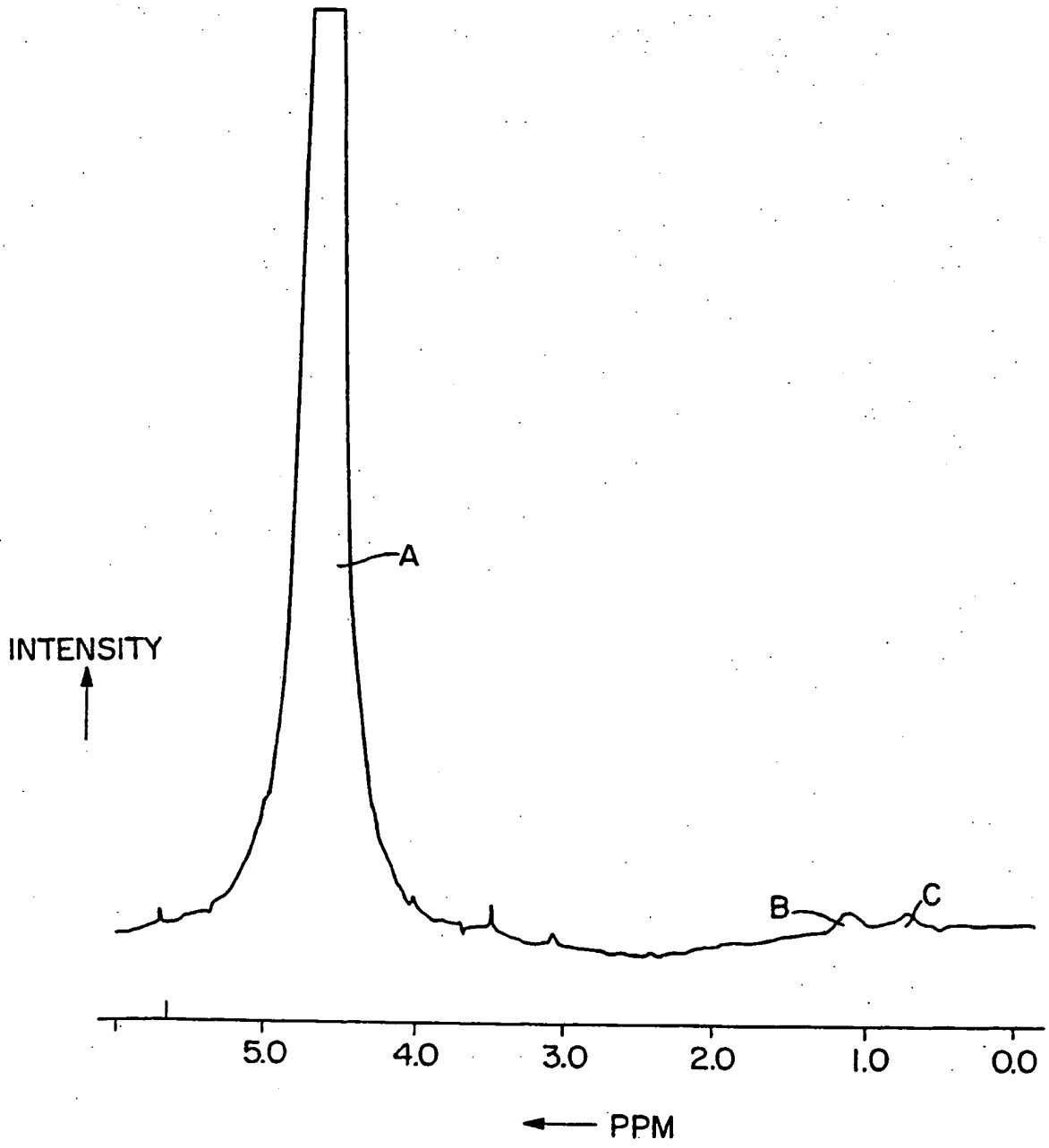


FIG. 3

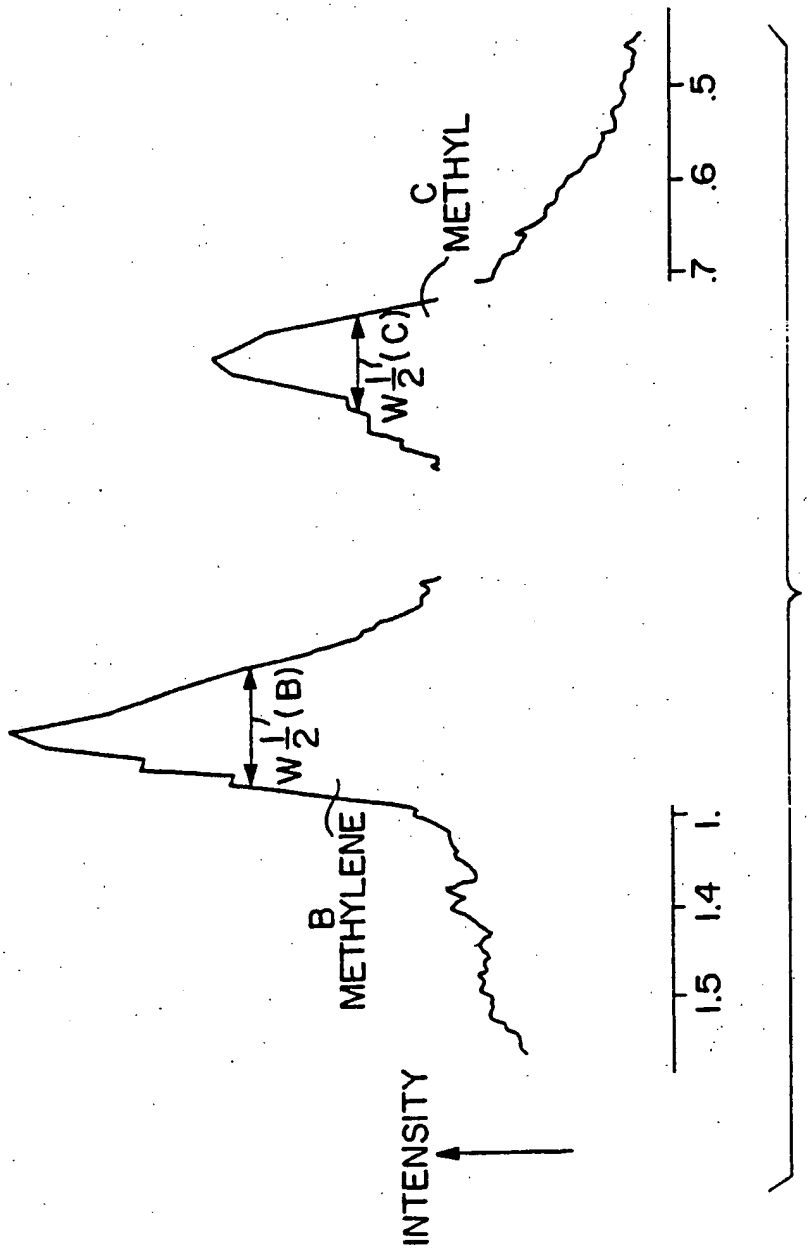
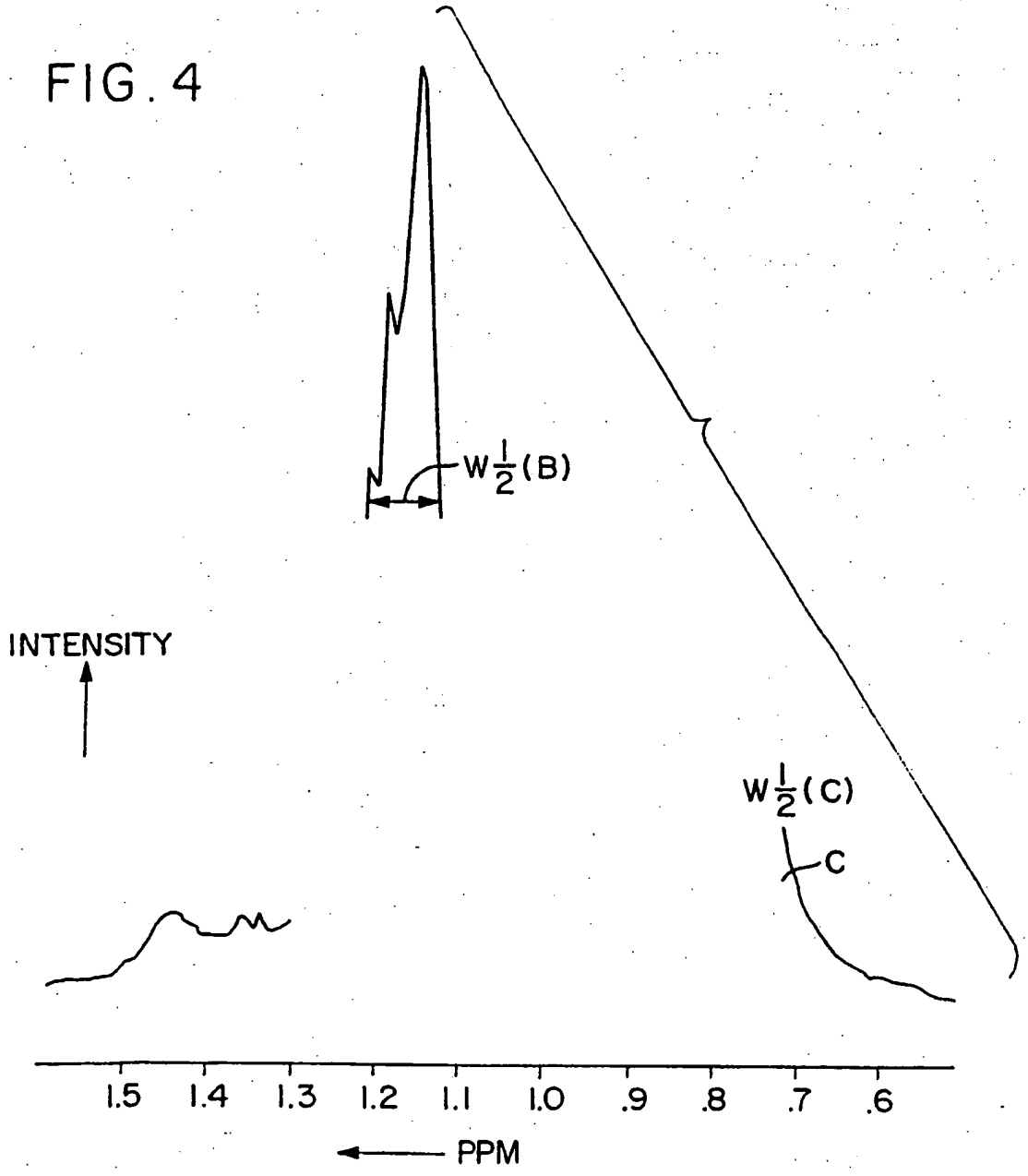

INTENSITY
B
METHYLENE
$W\frac{1}{2}'(B)$
C
METHYL
$W\frac{1}{2}'(C)$
1.5
1.4
1.
.7
.6
.5

FIG. 4



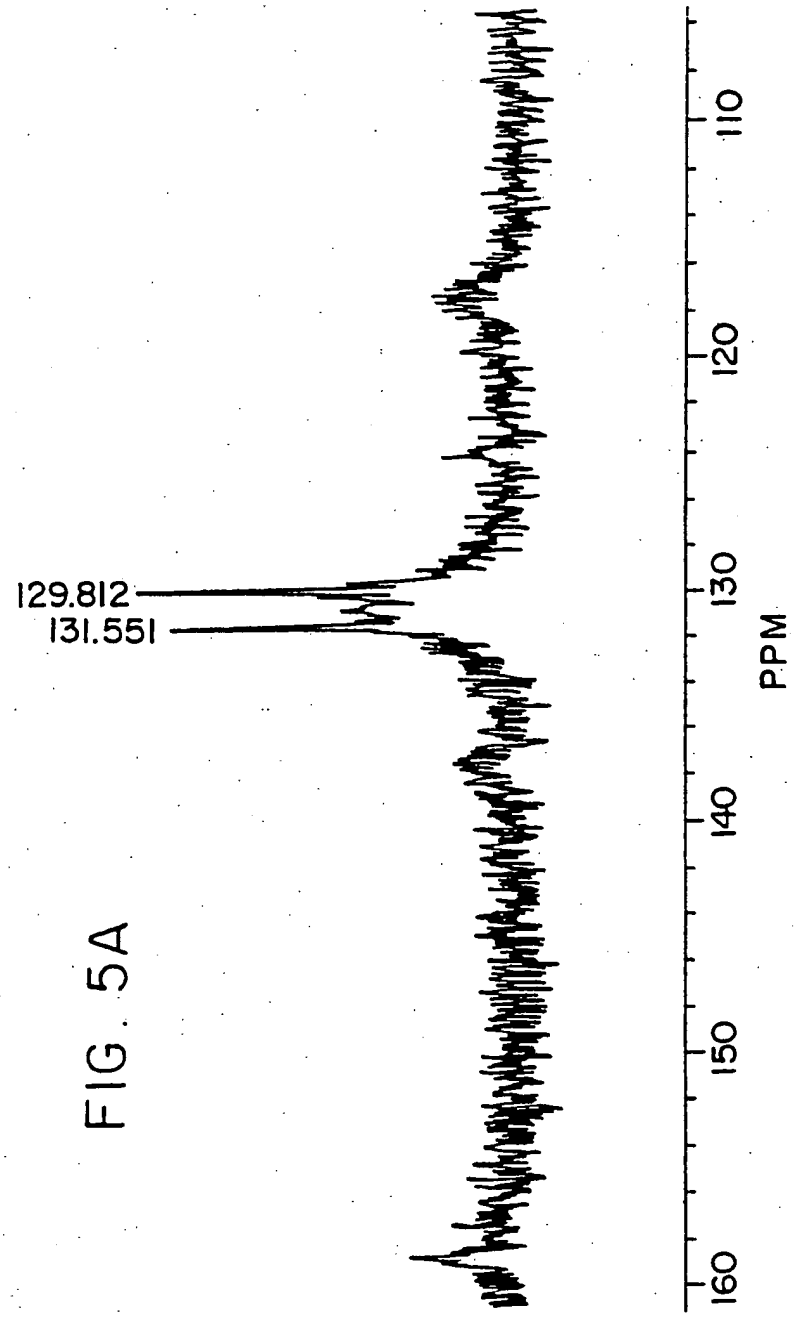


FIG. 5A

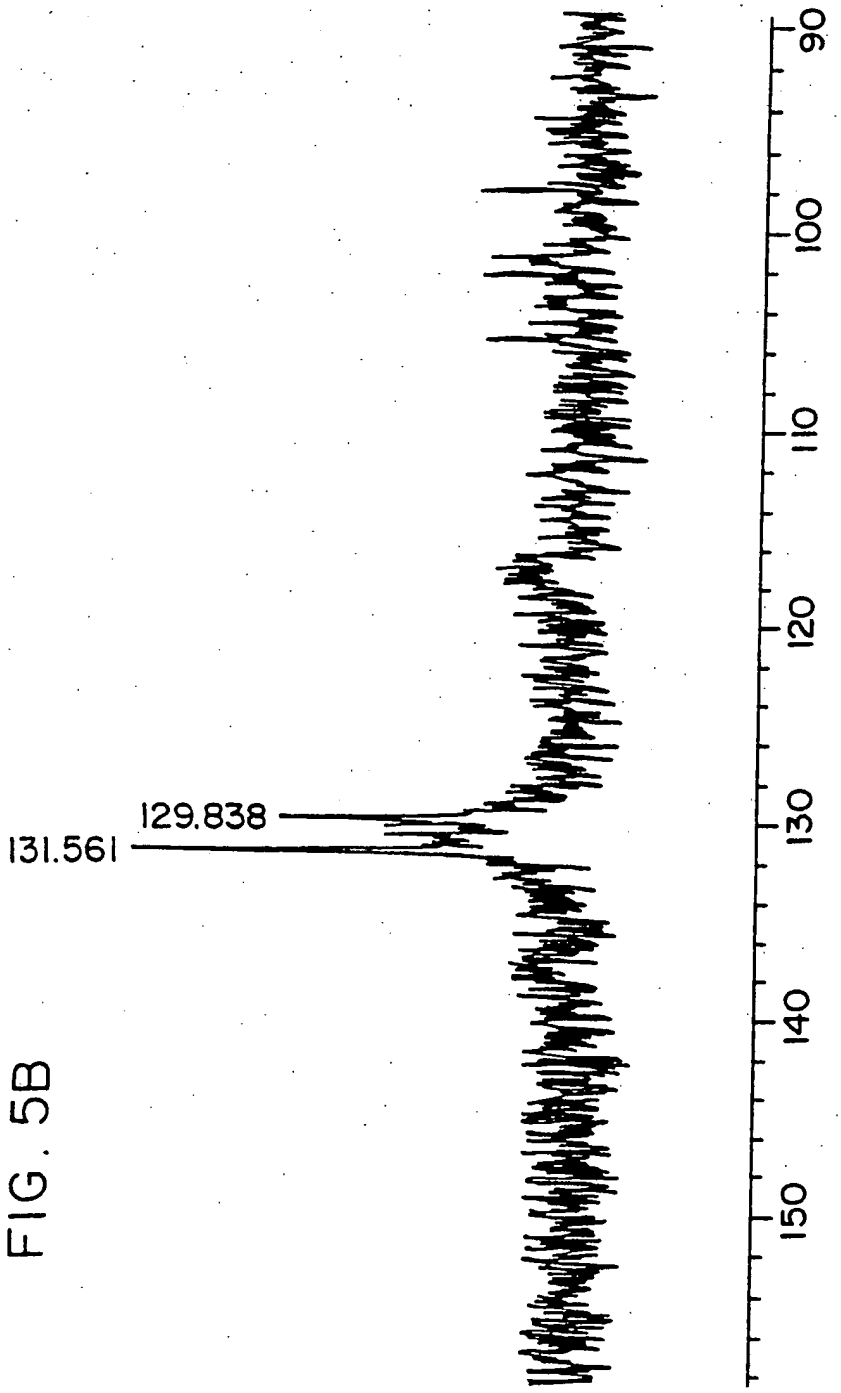


FIG. 5B

FIG. 6B

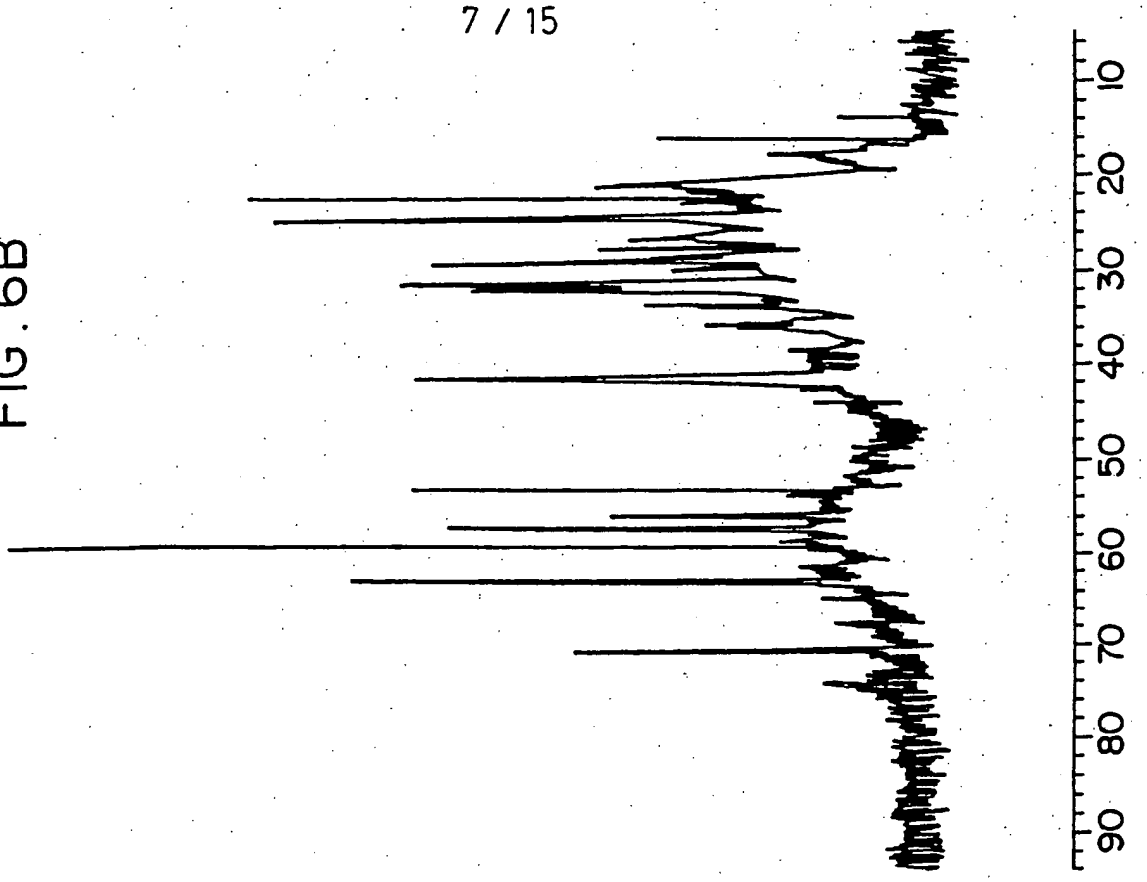
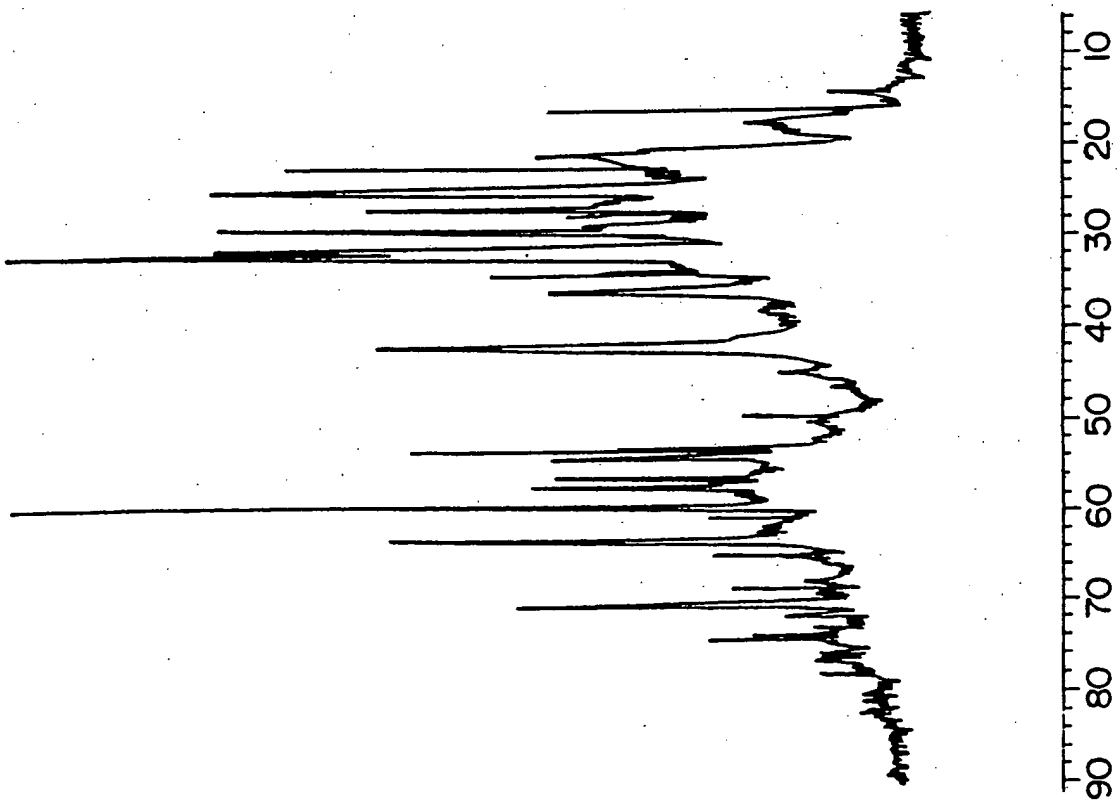


FIG. 6A



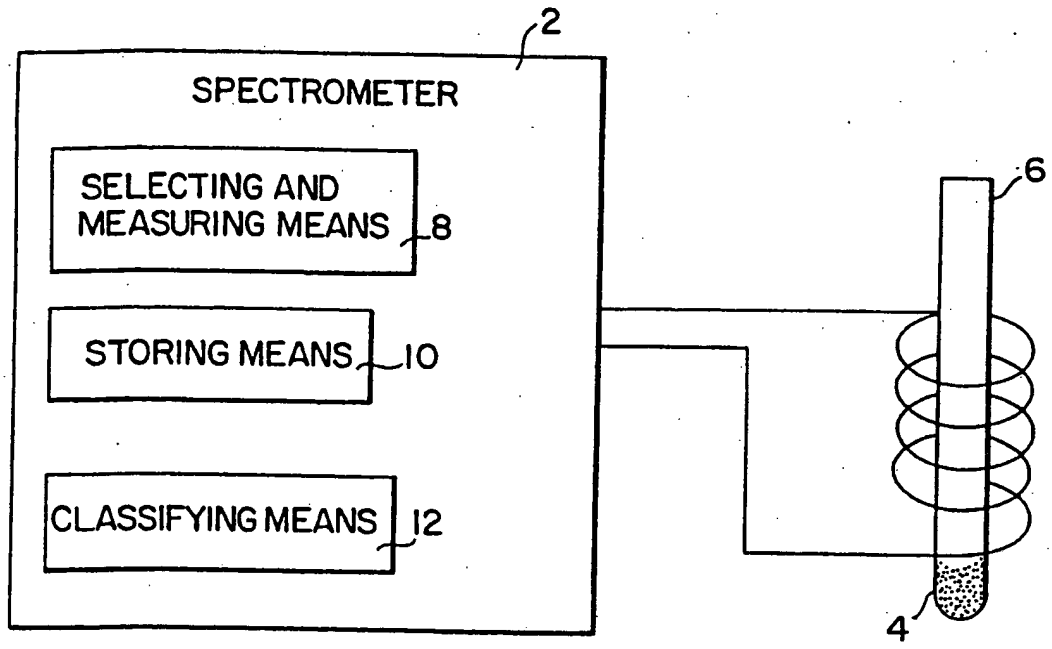
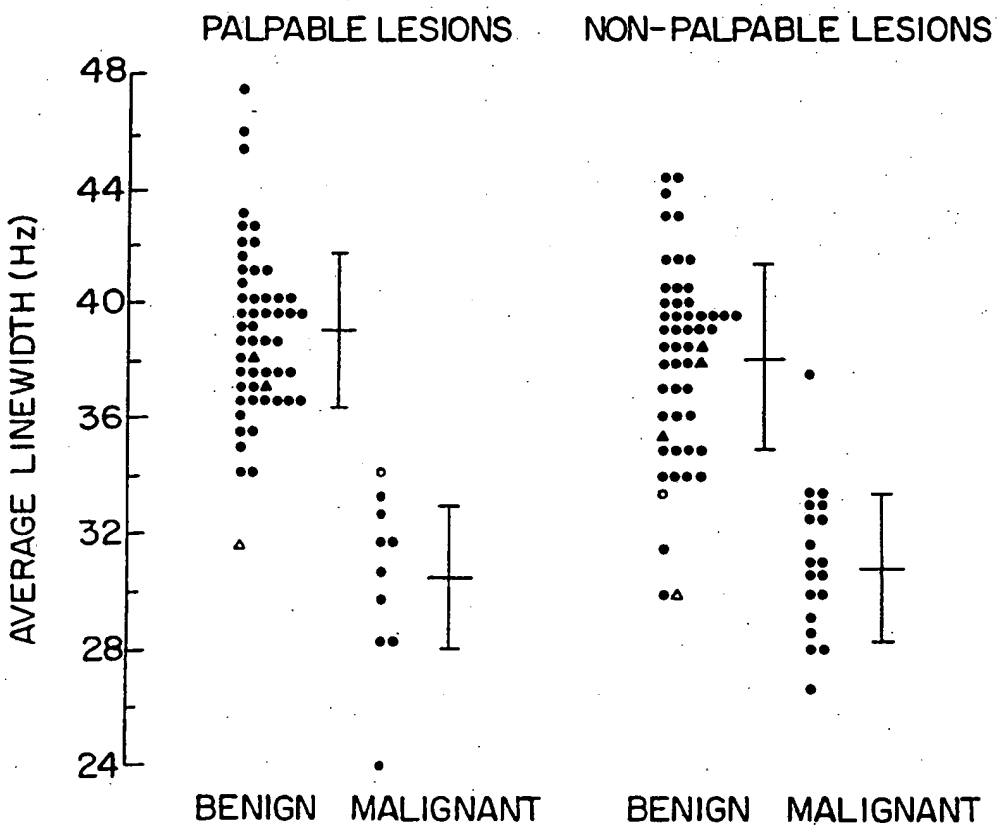

2
SPECTROMETER
SELECTING AND MEASURING MEANS
8
STORING MEANS
10
CLASSIFYING MEANS
12
6
4

FIG. 7

FIG.8


PALPABLE LESIONS
NON-PALPABLE LESIONS
AVERAGE LINEWIDTH (Hz)
48
44
40
36
32
28
24
BENIGN
MALIGNANT
BENIGN
MALIGNANT

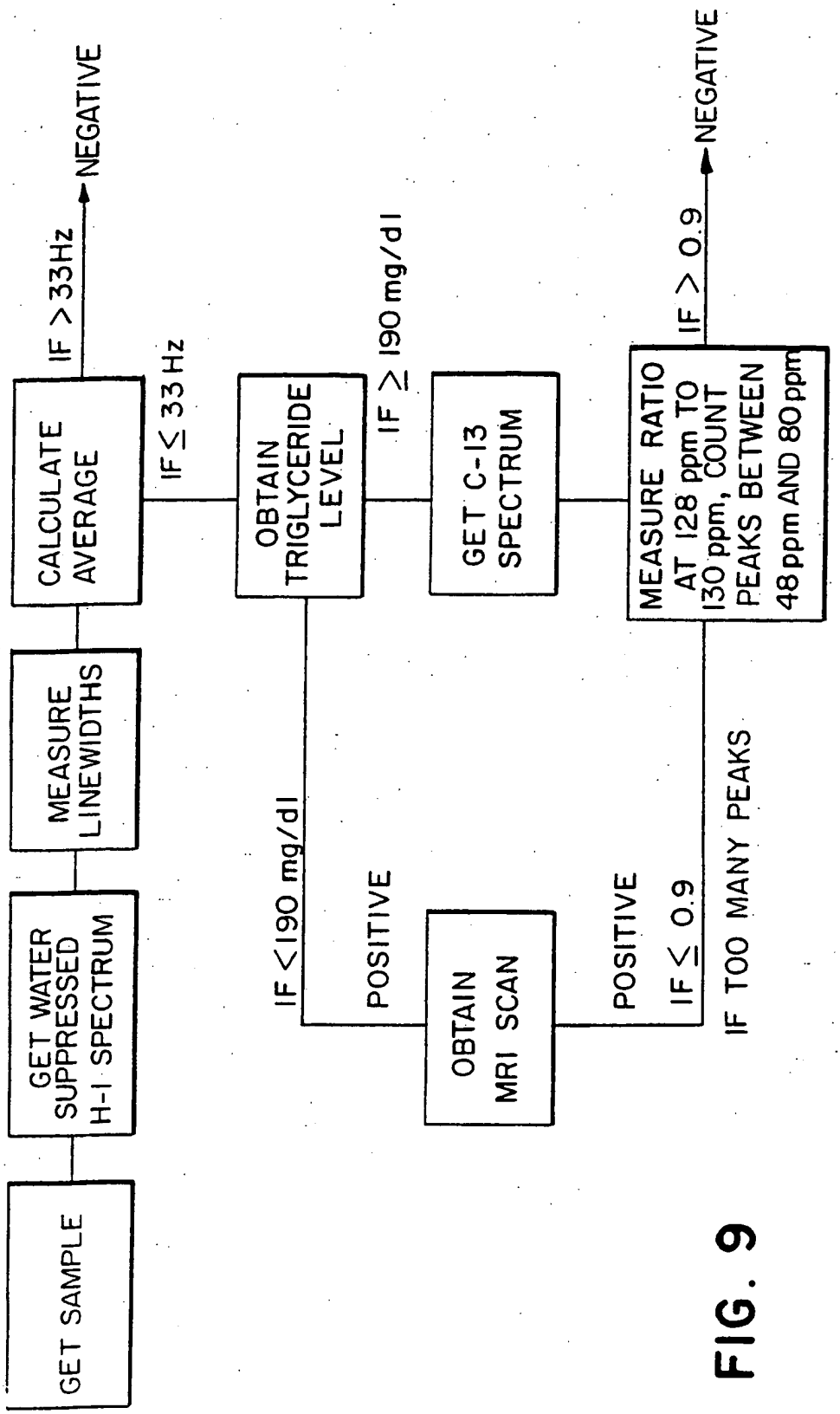
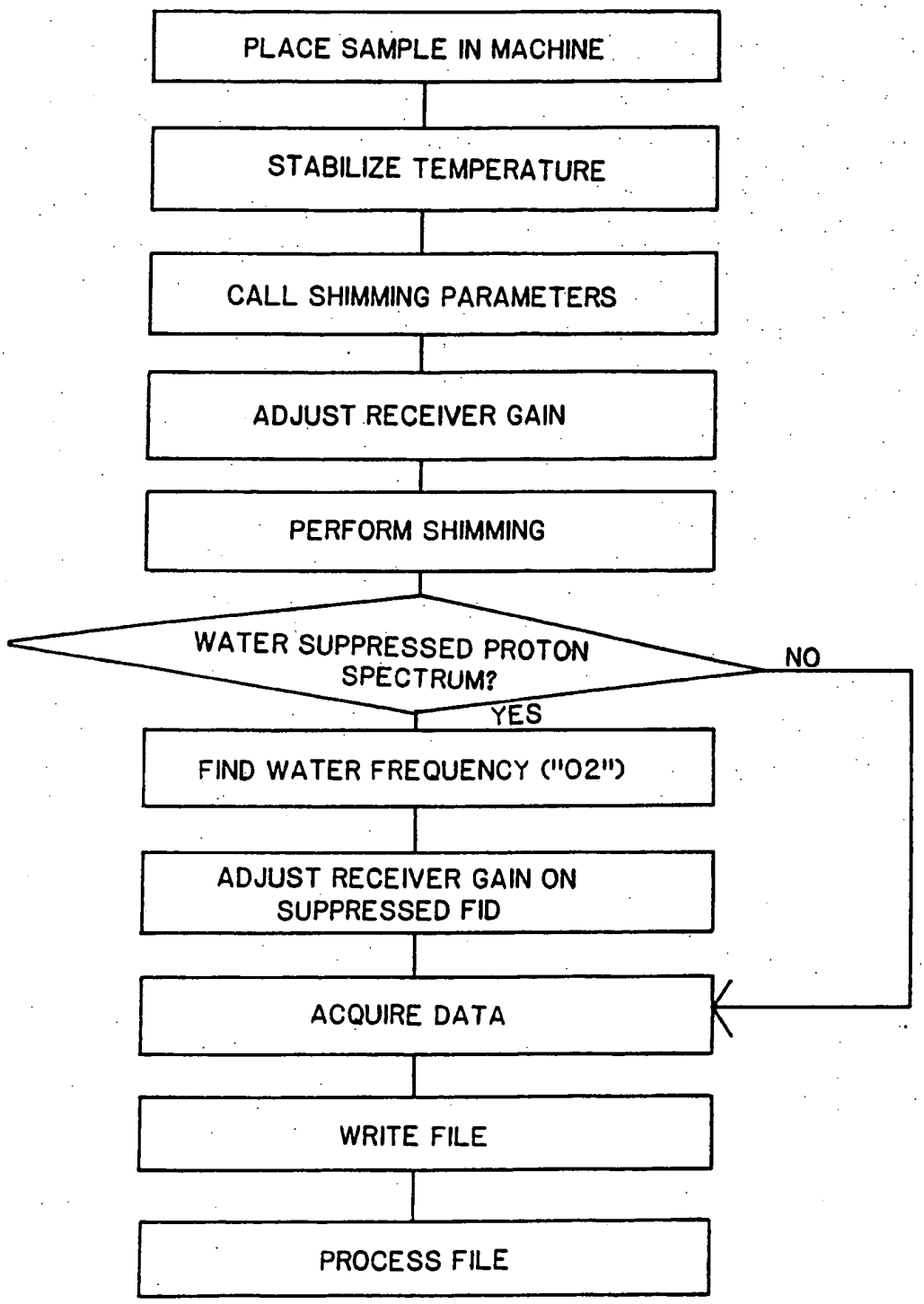

GET SAMPLE
GET WATER SUPPRESSED H-1 SPECTRUM
MEASURE LINEWIDTHS
CALCULATE AVERAGE
IF > 33Hz
NEGATIVE
IF ≤ 33 Hz
OBTAIN TRIGLYCERIDE LEVEL
IF < 190 mg/dl
POSITIVE
IF ≥ 190 mg/dl
GET C-13 SPECTRUM
OBTAIN MRI SCAN
MEASURE RATIO AT 128 ppm TO 130 ppm, COUNT PEAKS BETWEEN 48 ppm AND 80 ppm
IF > 0.9
NEGATIVE
IF ≤ 0.9
POSITIVE
IF TOO MANY PEAKS

FIG. 9

FIG. 10A


PLACE SAMPLE IN MACHINE
STABILIZE TEMPERATURE
CALL SHIMMING PARAMETERS
ADJUST RECEIVER GAIN
PERFORM SHIMMING
WATER SUPPRESSED PROTON SPECTRUM?
NO
YES
FIND WATER FREQUENCY ("02")
ADJUST RECEIVER GAIN ON SUPPRESSED FID
ACQUIRE DATA
WRITE FILE
PROCESS FILE

```
1     SX
2     RJ TUNE
3     II
44     D8
4     ZE
5     TU4
6     RJ FQSET
7     PJ FQSET
8     II
9     ZE
10    D1
11    P6:A
12    RGA=10
13    ZE
14    D1
15    P6:A
16    GO=14
17    FT
18    APK
19    PPD O2SET
20    PASC SETO2FRQ
21    O1CO
22    II
23    ZE
24    D6 S2 HG
25    P2:A
26    D7
27    P1:A DO
28    RGA=24
29    ZE
30    D6 S2 HG
31    P2:A
32    D7
33    P1:A DO
34    GO=30
35    WR #1
36    IF #1
37    LO TO 1 TIMES 120
38    EXIT
```

FIG. 10B

```
      ZE
1     SX
2     RJ TUNE
3     II
4     D8
5     ZE
6     TU4
7     ZE
8     RJ FQSET
9     PJ FQSET
10    II
11    LOCK
12    GO=12
13    WR #1
14    IF #1
15    ZE
16    LO TO 1 TIMES 120
17    EXIT
```

FIG. 10C

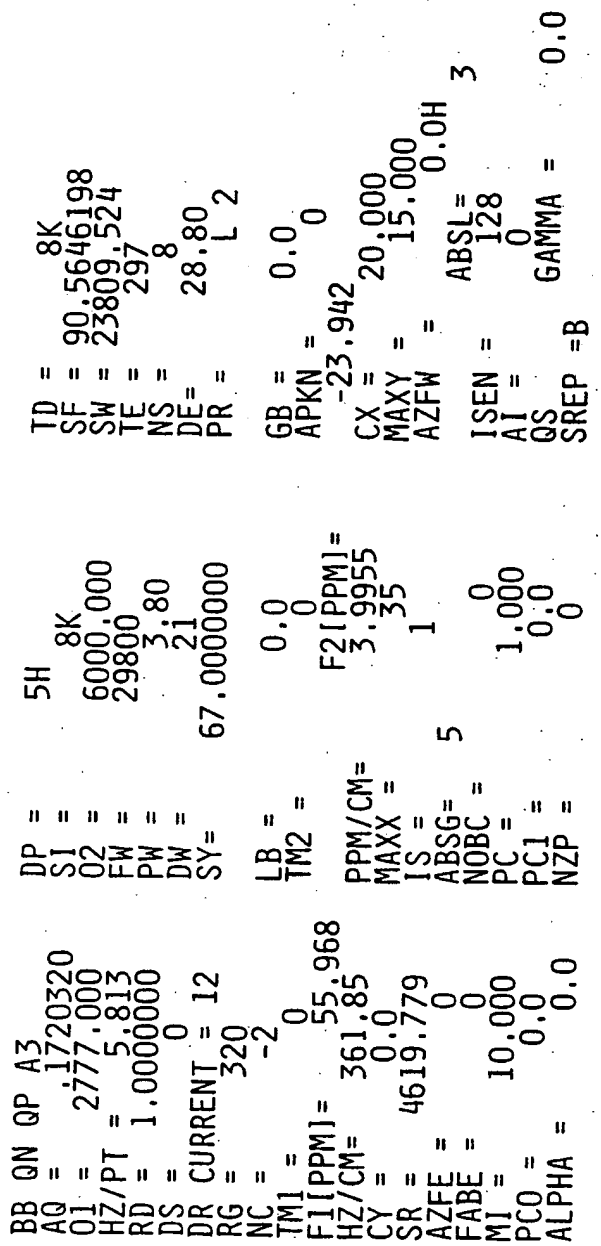

BB QN QP A3
AQ = 1720320
O1 = 2777.000
HZ/PT = 5.813
RD = 1.0000000
DS = 0
DR CURRENT = 12
RG = 320
NC = -2
TM1 = 0
F1[PPM]= 55.968
HZ/CM= 361.85
CY = 0.0
SR = 4619.779
AZFE = 0
FABE = 0
MI = 10.000
PCO = 0.0
ALPHA = 0.0
DP = 5H 8K
S1 = 6000.000
O2 = 29800
FW = 3.80
PW = 21
DW = 67.0000000
SY=
LB = 0.0
TM2 = 0
F2[PPM]= 3.9955
PPM/CM= 35
MAXX = 1
IS =
ABSG= 5 0
NOBC = 1.000
PC = 0.0
PC1 = 0
NZP =
TD = 8K
SF = 90.5646198
SW = 23809.524
TE = 297
NS = 8
DE= 28.80
PR = L 2
GB = 0.0
APKN = 0
-23.942
CX = 20.000
MAXY = 15.000
AZFW = 0.0H 3
ABSL= 128
ISEN = 0
AI =
QS GAMMA = 0.0
SREP =B

FIG. 10D

```
DO QN QP A3           DP =         7L               TD =        8K
AQ =     .5652480     SI =          8K              SF =360.1357816
O1 =   4800.000       O2 =       4752.665           SW =  7246.377
HZ/PT =    1.769      FW =        9100              TE =    297
RD =  1.0000000       PW =          2.00            NS =      0
DS =        2         DW =         69               DE=     88.80
DR CURRENT = 12       SY=    120.0000000            PR =        1 1
RG =        1
NC =       -9         LB =            0.0           GB =      0.0
TM1 =        0        TM2 =            0            APKN =        0
F1[PPM]=     14.065               F2[PPM]=              -6.032
HZ/CM=   361.88       PPM/CM=       1.0048          CX =      20.000
CY =       0.0        MAXX =          35            MAXY =      15.000
SR =   4619.779       IS =           1              AZFW  =          0.0H
AZFE =         0      ABSG=    5                               ABSL=       3
FABE =         0      NOBC =             0          ISEN =     128
MI =      10.000      PC =          1.000           AI =        0
PCO =       0.00      PC1 =          0.0            QS         GAMMA =      0.0
ALPHA =       0.0     NZP =            0            SREP =B
```

FIG. 10E

```
DO QN QP A3              DP =       12L            TD =         8K
AQ =      .5652480       SI =          8K          SF =360.1346198
O1 =    4800.000         O2 =      5778.700        SW =  7246.377
HZ/PT =    1.769         FW =       9100           TE =    297
RD =  0.0                PW =          0.0         NS =        8
DS =          2          DW =          69          DE=      88.80
DR CURRENT = 12          SY=    120.0000000        PR =         1 1
RG =          1
NC =         -2          LB =           0.0        GB =      0.0
TM1 =          0         TM2 =           0         APKN =       0
F1[PPM]=    14.065                 F2[PPM]=            -6.032
HZ/CM=    361.88         PPM/CM=      1.0048       CX =      20.000
CY =        0.0          MAXX =         35         MAXY =      15.000
SR =    4619.779         IS =           1          AZFW  =          0.0H
AZFE =         0         ABSG=    5                        ABSL=        3
FABE =         0         NOBC =          0         ISEN =      128
MI =      10.000         PC =          1.000       AI =        0
PCO =       0.00         PC1 =          0.0        QS          GAMMA =      0.0
ALPHA =       0.0        NZP =           0         SREP =B
```

FIG. 10F

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/00304

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5) A61B 5/055
U.S. CL. 128/653.2

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	128/653.1 324/307-309,312-318 436,64,71,173

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	New England Journal of Medicine, Vol. 322 No. 14 APRIL 1990, "NMR-Another Cancer Test Disappointed", pp 1002-3 SHULMAN	1-22
A	Science News, Vol. 137 APRIL 1990 NMR Test fails to Identify Cancer p 236	1-22
A	US,A, 3,789,832 05 FEBRUARY 1974 Damadian See entire document	1,10
A	US,A, 3,420,634 07 JANUARY 1969 Godsey See entire document	5,14

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 MARCH 1992	10 APR 1992
International Searching Authority	Signature of Authorized Officer
ISA/US	RUTH S. SMITH NGUYEN NGOC-HO INTERNATIONAL DIVISION

Form PCT/ISA/210 (second sheet) (Rev.11-87)